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Formulation and Characterisation of Ritonavir loaded Ethylcellulose Buoyant Microspheres

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Abstract
Microspheres are one of the novel drug delivery system and are formulated conveniently to obtain prolonged or controlled drug delivery to enhance the bioavailability and target drug to the specific sites. Ritonavir loaded ethylcellulose buoyant microspheres were formulated using ethylcellulose as rate retarding material. Buoyant microspheres were formulated by emulsion solvent diffusion method. Ritonavir buoyant microsphere were characterized and found that ethylcellulose polymer influenced in all the parameters evaluated. Incorporation efficiency was found to be high in all the formulations. Mean particle size were in the range of 195 to 342 µm, Buoyancy was found to be 63 to 72 %. *In vitro* release from all the formulations were found to be optimum.

Keywords : Buoyancy, Ethylcellulose, Microspheres, Ritonavir.

INTRODUCTION

Oral route has received the most attention and is quite successful due to the ease of administration, least aseptic constraints and ease of the manufacture of the dosage form. Microspheres are one of the novel drug delivery system and are formulated conveniently to obtain prolonged or controlled drug delivery to enhance the bioavailability and target drug to the specific sites. This also offers various advantages such as limiting fluctuation, decreasing dosing frequency, reducing side effects and improving patient compliance [1].

Drug absorption is unsatisfactory and highly variable between the individuals regardless of excellent *in vitro* release patterns [2]. Recent scientific literature has shown increased interest in novel drug delivery system that can be retained in the upper gastro intestinal tract for a prolonged and predictable period of time. One of the most feasible gastroretentive drug delivery is buoyant microspheres which is formulated to remain for the enhanced period of time in the upper gastro intestine tract there by reducing intersubject variability [3]. Indeed, the gastric emptying of a multiparticulate buoyant system would occur in a consistent manner with small individual variaitions. Since, each dose consists of many subunits, the risk of dose dumping is reduced [4].

Ritonavir is an oral antiretroviral drug from the class of protease inhibitor used in the treatment of acquired immunodeficiency syndrome (AIDS). It is widely prescribed in combination and its administration is indicated in adult patients with progressive or advanced stage [5,6]. The aim of the present study was to develop a buoyant microsphers of ritonavir using ethylcellulose as retarding polymer. The formulated buoyant microspheres were investigated for its yield, particle size, incorporation efficiency and *in vitro* dissolution studies.

MATERIALS AND METHODS

Ritonavir was generously supplied as a gift sample from Hetro drugs ltd., Hyderabad, India. Ethylcellulose was supplied by Colorcon asia pvt. ptd., (Mumbai, India). Polyvinyl alcohol purchased from S.D. fine chemicals, India. All solvents used were of analytical grade.

Preparation of Buoyant microspheres

Buoyant microspheres were prepared by the emulsion solvent diffusion technique [7-9]. The drug to polymer ratio used in the formulations consisted of 1:1 to 1:4 as presented in Table 1. Drug was dissolved in ethanol followed by adding the polymer to the above, isopropanol and dichloromethane were added one after another to the above mixture to get a clear solution. The above mixture was dropped slowly into the 0.5% polyvinyl alcohol aqueous solution. The mixture was stirred with a propeller type agitator at room temperature for 2 h at 300 rpm. The formed microspheres were carefully filtered, washed with water several times and dried at room temperature.

Table 1. Formulation of Ritonavir microspheres

Formulation	Ritonavir	Ethylcellulose
EC1	1	1
EC2	1	2
EC3	1	3
EC4	1	4

Production yield

The recovery of microspheres was determined by the weight ratio of the dried microspheres to the loading amount of the drug and polymer and it is expressed in terms of percentage as per the following equation.

$$\text{Production yield} = \frac{\text{total mass of microspheres}}{\text{total mass of raw materials}} \text{ x } 100$$

Particle size analysis

The formulated microspheres were characterized for its particle size by optical microscope [10]. The microscope was fitted with an ocular micrometer was calibrated with stage micrometer and 100 microspheres were examined, the mean particle size was calculated.

Drug loading and incorporation efficiency

The weighed quantity of microsphere powder (50 mg) was dissolved in methanol and the drug content was measured spectrophotometrically. The drug loading and incorporation efficiency were calculated by using the following equations [11].

$$\text{Drug loading (\%)} = \frac{M_{actual}}{\text{weighed quantity of powdered microspheres}} \times 100$$

$$\text{Incorporation efficiency (\%)} = \frac{M_{actual}}{M_{theoretical}} \times 100$$

Where M_{actual} is the actual drug content in the weighed quantity of powdered microspheres and $M_{theoretical}$ is the theoretical amount of drug in microspheres calculated from the quantity added in the formulation process.

***In vitro* buoyancy study**

50mg of microspheres were spread over the surface of USP paddle dissolution apparatus filled with 900 ml of 0.1 N HCl. The medium was agitated with a paddle at 100 rpm for 12 h. The floating and settled portions of microspheres were collected, they were separately dried and weighed individually. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remain floating and the total mass of the microspheres [12].

Fourier transform infra red (FT-IR) spectroscopic analysis

Spectra of drug, polymer and microspheres were recorded between the ranges of 4000 to 400 cm^{-1}. Samples were mixed uniformly with potassium bromide to obtain a uniform mixture [13]. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure and the pellet is used to record the spectrum.

Differential scanning calorimetry (DSC) Study

Differential scanning calorimetric analysis were performed on drug, polymer and drug loaded microspheres [14]. The instrument was operated under nitrogen purge at a range of 20 ml/min. Samples were placed in sealed aluminum pans. Thermograms were obtained at a heating range 10^0 C/min, over a temperature range of 25 – 400 ^{0}C. An empty aluminum pan was used as reference. Indium was used to calibrate the temperature scale and enthalphic response.

Scanning electron microscopy study

The morphology of the formulated microspheres was examined by scanning electron microscopy [15, 16]. The dry microspheres were mounted onto stub and sputter coated with gold particles in an argon atmosphere. Picture of microspheres were taken by random scanning of the stub.

***In vitro* dissolution study**

The ritonavir drug release from the microspheres was studied using USP paddle type dissolution test apparatus [17]. 100 mg of microspheres of all batches were carefully spread over the dissolution medium. 0.1N Hcl was used as dissolution medium maintained at 37 ± 0.5^0C. The dissolution apparatus was set to run at 100 rpm for 12 h. A sample of 10 mL was withdrawn from dissolution apparatus every hour and the samples were replaced with the fresh dissolution medium. The samples were suitably diluted with dissolution medium, filtered and analysed spectrophotometrically at 246 nm for the ritonavir content.

RESULTS AND DISCUSSION

Production yield

The percentage yield was found to be increased with increasing the polymer concentration shown in Table 2. It was found to be in the range of 70 to 86 %. Highest yield was obtained for ratio of drug to polymer was 1:4 as 86 %. This may be due to more amounts of solids in the solvent system used for the formulations [1].

Particle size analysis

The effect of polymer concentration on particle size were determined. The viscosity of the medium increases at higher concentration of polymers which resulted in the formation of larger particles. This may be due to the diminished shearing efficiency at more viscosities due to the polymer effect [18]. Larger particle size was found to be 342 µm for the formulation containing higher polymer EC4 as shown in the Table 2.

Drug loading and incorporation efficiency

Drug loading and incorporation efficiency were found to be satisfactory as evidenced from the Table 2. Microspheres with high incorporation efficiency was obtained with increasing concentration of polymers due to increasing ratio of drug to polymer. The high incorporation of drug in microspheres was believed to be due to poor solubility of drug in aqueous phase used during formulation process.

***In vitro* buoyancy study**

The in vitro floating test clearly showed that most of the microspheres were floating even after 12 h of testing. This may be attributed to their inherent low densities of the formulated microspheres. The buoyancy percentage was found to be in the range of 63 – 72 % as seen from the Table 2. The microspheres with the higher concentration of polymer were more buoyant than those with lower polymer concentration. This could be attributed to a decrease in density of particles with an increase in polymer concentration [19].

Table 2. Characterization of Ritonavir microspheres

Formulation	Yield (%) ± SD	Mean particle size (µm) ± SD	Drug loading (%) ± SD	Incorporation efficiency ± (%) SD	Buoyancy (%) ± SD
EC1	70.21 ± 2.06	195 ± 29.05	37.87 ± 1.18	75.74 ± 2.36	63.87 ± 7.79
EC2	79.77 ± 2.28	230 ± 31.58	26.57 ± 1.24	79.71 ± 3.72	65.79 ± 8.30
EC3	85.42 ± 1.69	274 ± 22.54	22.93 ± 0.52	91.74 ± 2.08	66.62 ± 4.81
EC4	86.55 ± 0.72	342 ± 24.71	18.45 ± 0.44	92.27 ± 2.22	72.90 ± 3.59

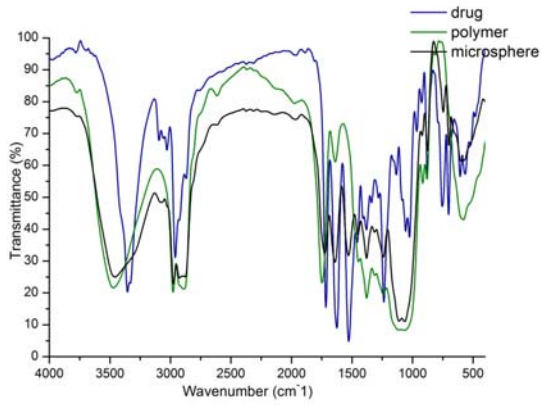

Figure 1. FT-IR spectra of Ritonavir, Ethyl cellulose, Microsphre

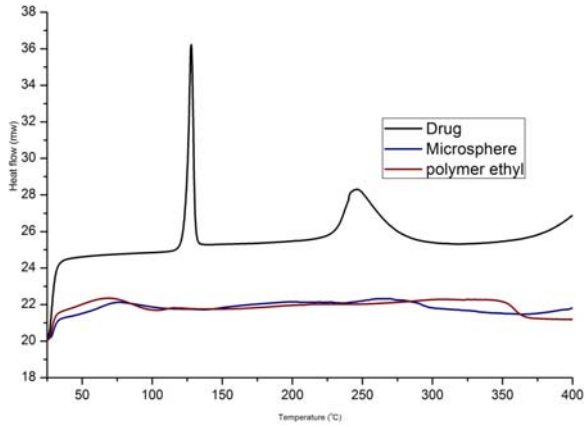

Figure 2. Differential scanning calorimetry thermograms

Fourier transform infra red (FT-IR) spectroscopic analysis

Drug polymer interaction was checked by the infra red spectrum of the formulated microspheres with the drug and polymer infra red spectrum were shown in Figure 1. Experimental observations were shown characteristics peak [20] for the drug at 3355 cm^{-1}, 2960 cm^{-1}, 1716 cm^{-1}, 1625 cm^{-1}, 1527 cm^{-1}. This supports that there is no interaction between the drug and polymer.

Differential scanning calorimetry (DSC) Study

To find out possibilities of any interaction between drug and polymer differential scanning calorimetry was carried out on the drug, polymer and microspheres the same shown in Figure 2. There were no interaction was seen on microspheres. No individual changes in the thermogram were observed indicating no interaction and the drug showed amorphous state in the microspheres [21].

Scanning electron microscopy study

The surface topography of the microspheres was examined by scanning electron microscope and shown in Figure 3. From the picture it is evident that it exhibited coarse surface. Pores were seen on the surface of the microspheres.

***In vitro* dissolution study**

In vitro dissolution studies of the formulated ritonavir buoyant microspheres were performed using dissolution test apparatus. It was found that ethyl cellulose polymer used for the formulation influenced the release profile of the drug from the microspheres [22, 23]. Formulation EC1 shown the maximum release of 92 % which contains lowest amount of polymer whereas, EC4 showed the lowest release of 80 % which contains highest amount of polymer as seen from the Figure 4. This clearly indicated the influence of ethylcellulose polymer over the release profiles.

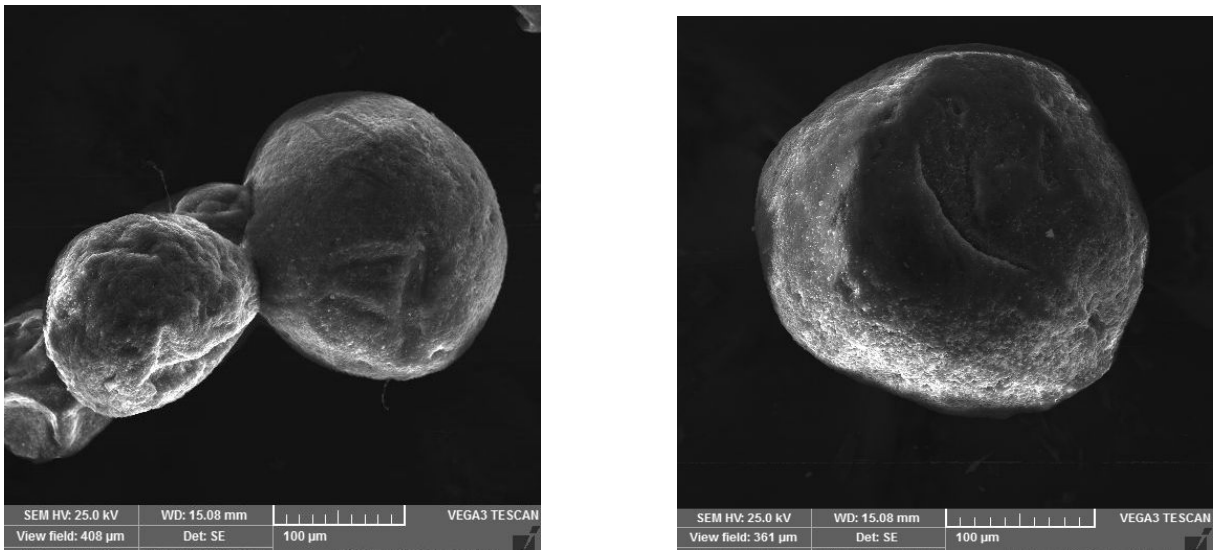

Figure 3. SEM of Ritonavir microspheres

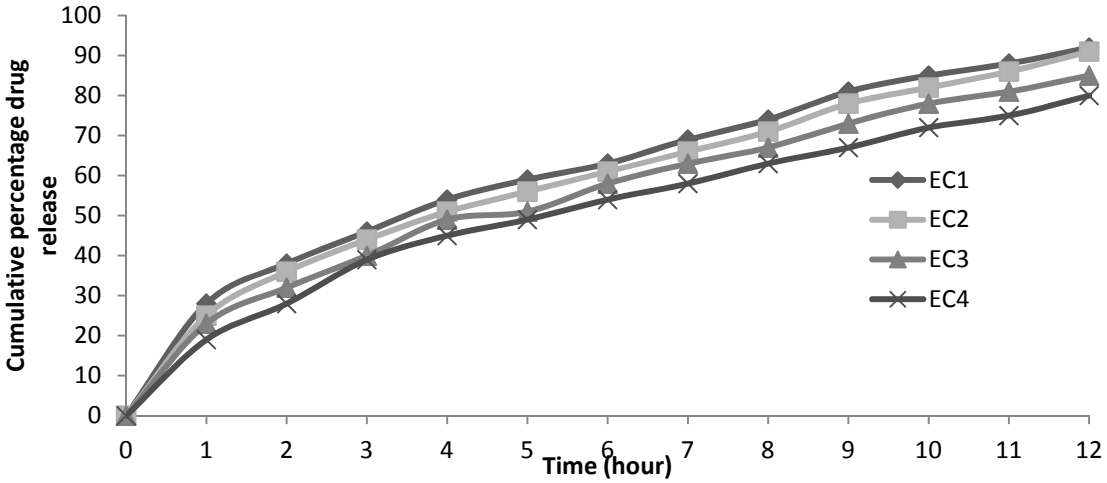

Figure 4. *In vitro* release studies of Ritonavir

CONCLUSION

Buoyant microspheres of ritonavir were successfully prepared by emulsion solvent diffusion method using ethylcellulose polymer. The concentration of the ethylcellulose influenced in all the evaluated parameters. The above findings demonstrated that ritonavir loaded ethylcellulose buoyant microspheres provide a convenient dosage form for achieving better release profiles and buoyancy properties.

REFERENCES

1. Subhash Vaghani, Vasanti, S., Kiran Chaturvedi, Satish, C.S., Jivani, N.P., *Pharm Dev Techno.* 2010, *15*, 154–161
2. Soppimath, K.S., Kulkarni, A. R., Rudzinski, W. E., Aminabhavi, T. M., *Drug Metab Rev*. 2001, *33*, 149-60.
3. Singh, B.N., Kim, K.H., *J. Control. Release*. 2000, *63*, 235–259.
4. El-Kamel, A. H., Sokar, M. S., Gamal, S. S., Naggar, V. F., *Int J Pharm.* 2001, *220*, 13-21.
5. Yekkala, R. S., Ashenafi, D., Mariën, I., Xin, H., Haghedooren, E., Hoogmartens, J., Adams E., *J Pharm Biomed Anal*. 2008, *48*, 1050-54.
6. Muñoz, M. J., Merino-Sanjuán, M., Lledó-García, R., Casabó, V. G., Máñez-Castillejo, F. J., Nácher, A., *Eur J Pharm Biopharm.* 2005, *61*, 20-26.
7. Kawashima, Y., Niwa, T., Takeuchi, H., Ito, Y., *J Pharm Sci*. 1992, *81*, 135 - 140.
8. Kawashima, Y., Niwa, T., Takeuchi, H., Ito, Y., *J Control Release*, 1991, 16, 279 - 290.
9. Lee, J. H., Park, T. G., Choi, H. K., *J Microencapsul.* 1999, *16*, 715-29.
10. Srivastava, A. K., Ridhurkar, D. N., Wadhwa, S., *Acta Pharm*. 2005, *55*, 277-85.
11. Yang, M., Cui, F. D., You, B., Fan, Y., Wang, L., Yue, P., Yang, H., *Int J Pharm*. 2003, *259*, 103-13.
12. Choudhury, P. K., Kar, M., Chauhan, C. S., *Drug Dev Ind Pharm*. 2008, *34*, 349-54.
13. Rastogi, R., Sultana, Y., Aqil, M., Ali, A., Kumar, S., Chuttani, K., Mishra, A. K., *Int J Pharm*. 2007, *334*, 71-77
14. Barakat, N. S., Ahmad, A. A., *J Microencapsul*. 2008, *25*, 31-45.
15. Mastiholimath, V. S., Dandagi, P. M., Gadad, A. P., Mathews, R., Kulkarni, A. R., *J Microencapsul.* 2008, *25*, 307-14.
16. Nepal, P. R., Chun, M. K., Choi, H. K., *Int J Pharm*. 2007, *341*, 85-90.
17. Choudhury, P. K., Kar, M., Chauhan, C. S., *Drug Dev Ind Pharm*. 2008, *34*, 349-54.
18. Rao, M. R., Borate, S. G., Thanki, K. C., Ranpise, A. A., Parikh, G. N., *Drug Dev Ind Pharm.* 2009, *35*, 834-42.
19. Umamaheswari, R. B., Jain, S., Tripathi, P. K., Agrawal, G. P., Jain, N. K., *Drug Deliv*. 2002, *9*, 223-31.
20. Sinha, S., Ali, M., Baboota, S., Ahuja, A., Kumar, A., Ali, J., *AAPS PharmSciTech*. 2010, *11*, 518-27.
21. Choudhury, P. K., Kar, M., *J Microencapsul*. 2009, *26*, 46-53.
22. Singh, B. N., Kim, K.H., *J. Control. Rel.* 2000, *63*, 235–259.
23. Costa, P., Lobo, M. S. J., *Eur. J. Pharm. Sci.* 1987, *39*, 39 - 45.